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FUNGAL CELL ADHESION MOLECULES IN *CANDIDA ALBICANS*

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Adherence of *Candida albicans* to host tissues is considered a crucial step in the pathogenesis of candidiasis. Using *in vitro* assays, it was demonstrated that the yeast - mycelium transition was an important phenomenon in the acquisition of adhesive properties. Proteins with MWs of 60, 68, 200 and > 200 kDa seemed to be involved in germ tube adherence to plastic surfaces. Likewise, recent investigations have revealed that *C. albicans* expresses on its surface receptors which interact with a wide variety of host proteins, particularly some extracellular matrix components like fibronectin, laminin and collagen. Plasmatic components, such as fibrinogen, iC3b and C3d, have also been proposed as mediators of adherence of *C. albicans*. Thus, by their reaction with laminin, fibrinogen and C3d, the mannoproteins of 68 and 60 kDa demonstrated multiple biological activities. Proteins of similar MWs were detected as C3d and iC3b receptors, the latter showing similarities with the neutrophil CR3. Based upon the antigenic, structural and functional homologies between the candidal receptors and mammalian integrins, it was postulated that these fungal cell adhesion molecules (F-CAM) are members of the integrin family. Interactions with host proteins and molecular mimicry of mammalian adhesion molecules may be a fertile area for further research.

INTRODUCTION

The phenomenon of microbial adherence is an important pre-requisite to many essential cellular functions and is now widely considered as one of the major events in the expression of the pathogenic potential of many microorganisms. Adherence implies the recognition of the host tissues by the pathogen and requires the presence of molecules on the microbial surface, known as adhesins, which are complementary to others on the surface of the host cells known as ligands. Adhesins are membrane or cell-wall glycoproteins which act as receptors for plasmatic or extracellular matrix (ECM) components possessing adhesive properties, like fibronectin, laminin, fibrinogen, collagen or complement components.

Study of the adherence of yeasts of the *Candida* genus has been primarily focused on the effects of factors, such as temperature, pH, culture medium, cell type, species or strain, on adherence to the host cells (for a review, see Douglas, 35). These studies have shown that the adherence of *Candida albicans* is largely influenced by the yeast-mycelium transition. This phenomenon of dimorphism, which appears to be closely associated with pathogenicity, results in reorganization of the cell wall at the molecular level. At least two changes in the wall are important in the acquisition of adhesive properties by *C. albicans*: i), the development of a fibrillar mannoprotein layer associated with the outer region of the wall; and ii), the existence of germ tube specific mannoproteins, expressed primarily at the cell wall surface (Table 1). These modifications allow the fungus to acquire high capacities of attachment, with important implications in tissular colonization. Indeed, a role for

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TABLE 1. - Proteins (P) or mannoproteins (MP) specific to the germ tube surface.

References	Molecular weights
Ahrens et al., 1983 (1)	P_{31}, P_{40} and P_{75}
Sundstrom and Kenny, 1984 (58)	P_{25}, MP_{155} and MP_{200}
Sundstrom and Kenny, 1985 (59)	MP_{155} and MP_{200}
Ponton and Jones, 1986 (46, 47)	P_{19} and MP_{250}
Sundstrom et al., 1987 (60)	MP_{200}
Sundstrom et al., 1988 (61)	MP_{200}
Casanova et al., 1989 (8)	MP_{180} and MP_{200}
Leusch, 1989 (37)	MP_{85} and MP_{67}
Ollert and Calderone, 1990 (43)	P_{55} and MP_{60}

mannoproteins in mediating yeast attachment to epithelial cells has been established. Mannoprotein adhesins, which appear to originate from the fibrillar outer layer, have been isolated, but not characterized (11, 42). However, pretreatment of crude mannoprotein adhesins with proteolytic enzymes destroyed their ability to inhibit adhesion, indicating the involvement of the protein portion in the attachment process (11). Lectin-like properties have also been reported for yeast mannoproteins, suggesting that glycosides containing L-fucose (12, 57), D-mannose (9, 12, 51), or N-acetyl-D-glucosamine (10, 12, 54) can function as epithelial cell receptors for *C. albicans*. Other studies have suggested that lipids play a role in *C. albicans* adherence to epithelial cells (18, 19, 29). Indeed, glycosphingolipids of the host cell surface, and particularly ceramide dihexosides, like lactosylceramide, may play a role in yeast adhesion (29).

During the last few years, the present authors and other groups have been greatly interested in the molecular aspects of *C. albicans* adherence. Particular attention has been focused on the role of plasmatic or matrix host proteins. *In vitro* studies have shown that certain host proteins with adhesive properties can be recognized by the yeast. So, iC3b and C3d, as well as fibrinogen, fibronectin and laminin, have been proposed as host cell receptors for *C. albicans*. Some candidal adhesins have also been identified.

In this article, the interactions with these host proteins will be reviewed. The topics of current investigations that will be discussed include: (i), adherence of *C. albicans* to plastic and the principal approaches used to study the fibrillar adhesins; (ii), adherence to basement membrane and ECM glycoproteins; (iii), interactions with fibrin platelet clots and fibrinogen; (iiii), interactions with complement components. To facilitate the understanding of these interactions, the structure, function and physiology of these host proteins will be discussed briefly. For further details, extensive literature is available.

Adherence to plastic:

Candida albicans can adhere to various biomaterials, such as contact lenses, urinary catheters or prosthetic devices. Invasion of the human host can occur by vascular dissemination of yeasts attached to intravenous catheters. Chronic atrophic candidiasis is a common problem in elderly denture wearers. Despite its potential relevance to pathogenicity, fungal adherence to plastic surfaces has received relatively little attention (for a review, see Rotrosen et al., 48).

The ability of yeast to adhere to various inert surfaces has recently been examined by Klotz et al. (33). *Candida albicans* adhered poorly to pyrex glass, poly(ethyleneterephthalate) and poly(methylmethacrylate); adherence to polystyrene and poly(tetrafluoroethylene) was considerably greater. Electrostatic forces and hydrophobicity have been reported to be responsible for such interactions (23, 24,, 33). Cell surface hydrophobicity (CSH) has been shown to be an important property of *C. albicans*, which allows it to bind to plastic. Expression of CSH is a dynamic process which decreases prior to the appearance of morphological evidence of germ tube formation. Unfortunately, CSH has not been

found to correlate well with yeast adherence to human epithelial cells (24), indicating that, if CSH is involved in this type of adherence, it is not the predominant mechanism. Since studies on CSH have suggested that the surface components responsible for hydrophobicity are cell wall proteins, we developed a reliable and easily quantifiable adherence assay on plastic in order to identify *C. albicans* adhesins (Fig. 1).

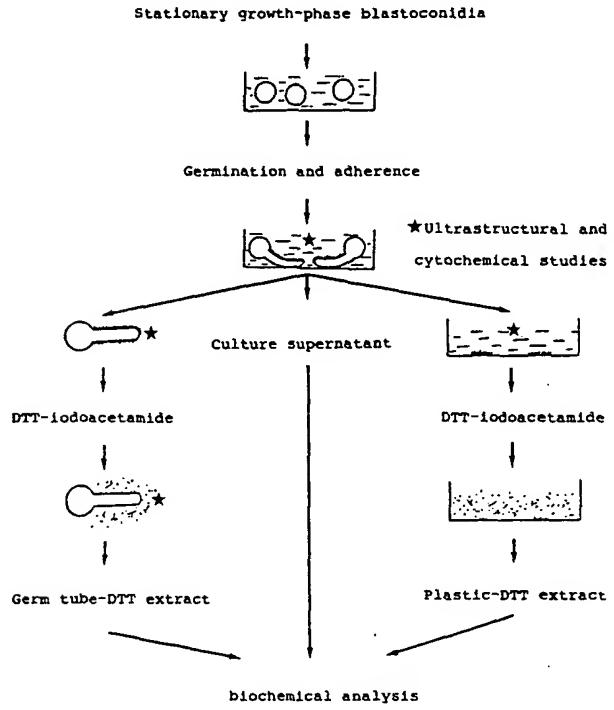


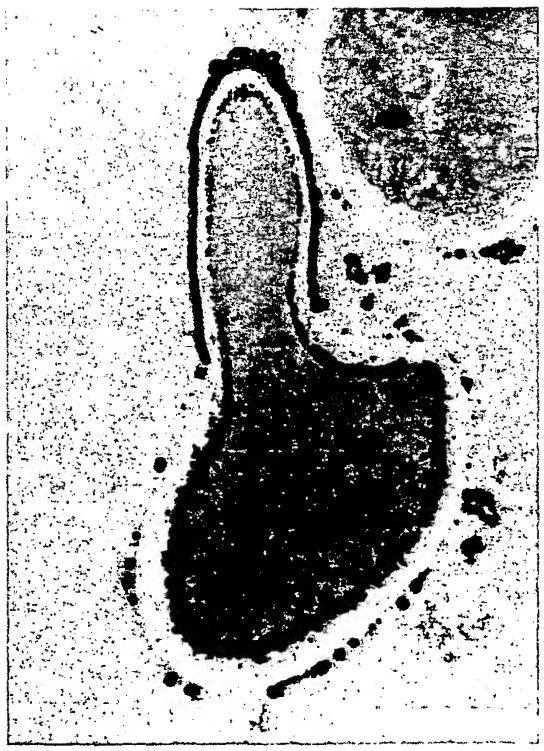
Figure 1. - Schematic representation of the methodology used for the study of *C. albicans* adherence to plastic.

Experimental approaches:

Stationary growth-phase blastoconidia produced in synthetic liquid medium were inoculated into medium 199 and incubated at 37°C for 3 hours in biologically neutral polystyrene Petri dishes. Under these conditions, a strong correlation was observed between germination and adherence to plastic. This suggests that germ tube formation is accompanied by biochemical changes of the cell wall which lead to the expression of adhesive proteins at the hyphal surfaces. Rearrangement of the cell-surface mannoproteins during germination was also studied by electron microscopy, and we attempted to visualize the fungal structures associated with adherence. Finally, molecules remaining attached to the plastic were analyzed by SDS-PAGE and fluorography after biosynthetic labeling.

1. *Dynamic changes of the cell wall surface associated with germination and adherence:*

The distribution of mannoproteins at the cell wall surface of *C. albicans* was analyzed during the process



Figures 2. and 3. - Rearrangement of the cell surface during germination revealed by electron microscopy. Detection of concanavalin A binding sites (Fig. 2) and of acid phosphatase activity (Fig. 3). Note the intense labeling of the germ-tube surface, contrasting with the weak reaction of the surface of the mother cell. (Fig. 2, x 15,000; Fig. 3, x 10,000).

of germination in conditions favouring adherence of germ tubes to a plastic matrix as described above (62). Three cytochemical methods allowing the detectio of cell wall mannoproteins (distribution of concanavalin A binding sites, of anionic sites, and of the enzyme acid phosphatase) were used. The results indicated that, during germination, density and distribution of the cell wall surface mannoproteins were affected (Figs. 2 and 3), and that a spatial and temporal reorganization of the cell wall occurred. Intense labeling was observed at the surface of resting blastoconidia. In contrast, as soon as germ tube formation was complete, labeling intensity decreased dramatically at the surfaces of mother cells, and the hyphal surfaces appeared strongly stained. Thus, it seems that reorganization of the cell wall during germination implies enzymatic degradation of the mannoproteins present at the yeast surface and expression of new components on the hyphal surface. Together with presentation of molecules possessing multifunctional properties (see below), this phenomenon could explain why the process of dimorphism must be considered as a crucial step in the expression of the pathogenicity of *C. albicans.*

2. *Ultrastructural and molecular study of fibrillar adhesins:*

Using electron microscopy and concanavalin A-gold labeling, we demonstrated that germ tubes adhering to plastic had developed an additional outermost fibrillar layer containing mannose residues, and that fibrils formed connections with the plastic at the site of cell-substratum contact (Fig. 4) (63). To determine if fibrils promote attachment to plastic, it was necessary to demonstrate that cell wall material isolated from adhesion plaques in plastic Petri dishes expressed fibrillar adhesins. This was realized using the following experiments: (i), extra-cell wall mannoproteins were detected at the sites of cell substratum contact by concanavalin A-sensitized latex microspheres. (ii), by electron microscopy, we visualized fungal fibrils retained on the plastic at sites of germ-tube substratum contact (Fig. 5). (iii), after

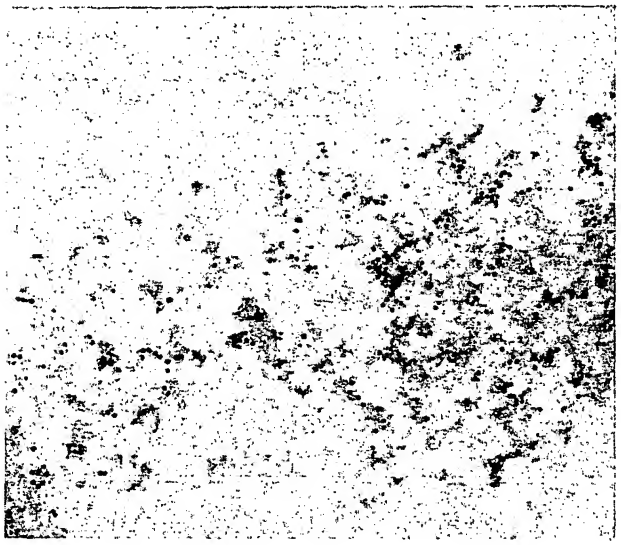


Figure 5. - Fibrils retained on the plastic after removal of the adherent germ tubes. Detection by electron microscopy using concanavalin A-gold (x 30,000).

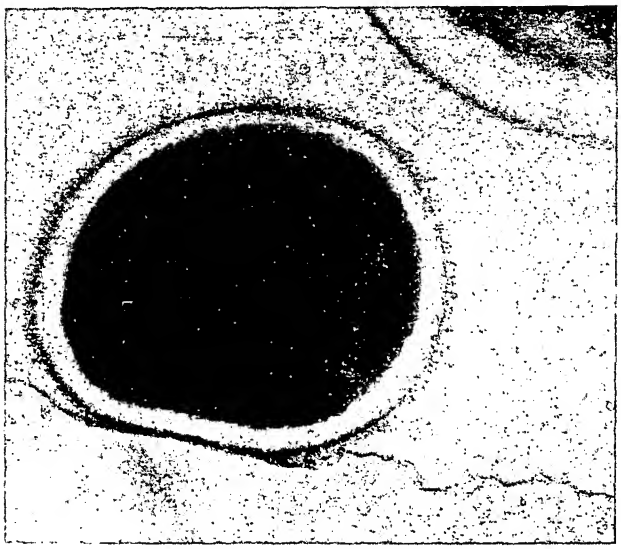


Figure 4. - Transverse section of a germ tube adhering to a plastic Petri dish. At the site of germ tube-substratum contact, the cell wall is closely apposed to the plastic surface. Note the distinct fibrogranular layer all around the germ-tube cell wall, which is not apparent in the parent blastoconidia (x 25,000).

biosynthetic labeling with L-[35_S] methionine, the material extracted by dithiothreitol-iodoacetamide treatment of the Petri dishes after mechanical elimination of the adhering germ tubes was electrophoresed and fluorographed. Four cell wall proteins with MWs of 60, 68, 200 and > 200 kDa were detected. Compared to the large variety of polypeptides observed in the germ tube-DTT extract as in the culture supernatant, the detection of these four proteins from the adhesion plaques clearly demonstrated their involvement in germ tube adherence to inert surfaces. Interestingly, as you will see below, the components of 60 and 68 kDa act as receptors for some host proteins like laminin, fibrinogen and complement, suggesting that they might play a role in the induction of adherence to host cells. Together, the adhesion of germ tubes to untreated plastic surface, and the detection of mannoprotein adhesins at sites of yeast-substratum contact must be now taken into account in future experiments using host protein-coated plastic for studies of *C. albicans* adherence.

Adherence to basement membrane and ECM components:

Evidence of the importance of basement-membrane and/or ECM components in the phenomenon of *C. albicans* adherence is based on several observations. Using cultured bovine endothelial cells, Klotz et al. demonstrated that adherence took place at the intercellular junctions and at portions of the basement membrane-like matrix left exposed after contraction or removal of the cells (31, 35). *In vitro*, basement membranes underlying epithelial and endothelial cells are not normally exposed, except in the fenestratred endothelia of kidney glomeruli, which are frequently involved in candidosis. These membranes may also be exposed after tissue damage, such as that resulting from catheterization or the use of chemotherapeutic drugs. Among the constituents of basement membrane and ECM, laminin, fibronectin and type IV collagen seemed to be good candidates for mediators of *C. albicans* adherence.

Figure 6. - Binding of laminin to *C. albicans* germ tube producing mycelium, visualized by immunofluorescence (x 1,500).

Interaction with laminin:

Recent evidence of the role of laminin in cell adhesion has led us to consider the existence of *C. albicans* receptors for this major structural glycoprotein of basement membranes. Indeed, laminin mediates the adhesion of several cell types *in vitro:* endothelial cells, leukocytes and metastatic tumour cells, but also various microorganisms like *Escherichia coli, Staphylococcus aureus, Treponema pallidum* or *Trichonomas vaginalis* (for review, see Campbell and Terranova, 7).

This hypothesis was first confirmed by immunofluorescence assay and electron microscopy (5). These two techniques, used with soluble laminin extracted from the Englebreth - Holm - Swarm sarcoma tumour, showed that binding of this protein was closely related to germination. Neither resting blastoconidia nor mother cells of germ tubes bound laminin, whereas germ tubes and mycelium were intensely labeled (Fig. 6). Furthermore, electron microscopy showed that labeling was associated with the outermost fibrillar layer of the hyphal surface.

The use of ^{125}I-radiolabeled laminin demonstrated that binding sites on the germ-tube surface present characteristics of receptors (5). Binding was rapid and saturable, suggesting the presence of a limited number of binding sites. Moreover, competitive experiments, performed by coincubation of the cells with increasing concentrations of radiolabeled laminin and a 100-fold molar excess of unlabeled ligand, demonstrated the specificity of the binding. Finally, Scathard plot analysis of the data obtained revealed the presence of a unique class of receptor sites (about 8,000 per cell) with a dissociation constant (K_d) of 1.3 x 10^{-9} M.

Since binding took place on fibrils of the outermost cell-wall layer of germ tubes, material extracted by DTT-iodoacetamide treatment was expected to contain the candidal receptors. Therefore, their identification was realized by SDS-PAGE and ligand blotting (5). Of the various polypeptides present in the germ tube extract, only two components of 68 and 60-62 kDa were detected by incubation of the nitrocellulose strips with laminin.

More recently, Klotz (32) investigated the implication of the laminin fixation in the adherence of *C. albicans*. The fungus was shown to adhere avidly to laminin-coated surfaces. In addition, attachment was inhibited in the presence of soluble laminin, or by prior incubation of the immobilized protein with specific polyclonal antibodies. In contrast, coating of plastic with various proteoglycans which are abundant in the extracellular matrix, resulted in a decrease of the attachment abilities of *C. albicans*. These results suggest that *C. albicans* germ tubes possess receptors for laminin whose characteristics (density, dissociation constant, molecular masses and biological properties) are similar to those described for laminin receptors on mammalian cells, particularly metastatic tumour cells.

Interaction with collagen and fibronectin:

Other basement membrane or ECM components may also mediate the adherence of *C. albicans*. Type IV collagen has been shown to enhance the attachment of the yeast to plastic surfaces (32). Adherence of *C. albicans* to collagen-coated surfaces, as opposed to attachment to immobilized laminin or fibronectin, was not inhibited by rabbit specific polyclonal antibodies. More recently, Klotz and Smith (36) have demonstrated that adherence of *C. albicans* to subendothelial ECM or to type IV collagen is inhibited by RGD-peptides.

Interactions with fibronectin have been more fully documented. This large glycoprotein could act as host-cell receptor for *C. albicans*. Fibronectin is present in soluble form in plasma and various other body fluids, and is associated, in an insoluble form, with various human cells (65). Its plasma concentration rises significantly in pregnancy and in diabetes mellitus, both of which are predisposing factors for candidosis.

First, Skerl et al. (55), using different yeast species, showed that *C. albicans* and *C. tropicalis* adhered more strongly to fibronectin-coated surfaces than did less pathogenic or non-pathogenic species, i.e. *C. krusei* and *Saccharomyces cerevisiae*. The incidence of *in vitro* binding of fibronectin to various *Candida* species also correlated with the ability of the organisms to cause endocarditis in rabbits after an experimental valvular trauma (53). Secondly, fibronectin has been detected at the surface of buccal or vaginal epithelial cells, and pretreatment of *C. albicans* blastoconidia with soluble fibronectin reduced their adherence to epithelial cells (30, 55).

Fibronectin-coated plastic enhanced the adhesion of yeasts and this attachment was inhibited by soluble fibronectin. Adherence to immobilized fibronectin was also inhibited in the presence of soluble mannans or by pretreatment of the yeasts with proteolytic enzymes, such as chymotrypsin, papain or pronase (55). Interestingly, mannans have been shown to block the adherence to fibrin-platelet clots (40) or to buccal (51) and uro-epithelial cells (9). However, the candidal receptors for fibronectin have not yet been identified. They are probably different from those for laminin, fibrinogen and C3 fragments since they are present in all morphological stages of the fungus.

Interactions with fibrin-platelet clots and fibrinogen:

Previous studies have demonstrated a close apposition of *C. albicans* yeasts and platelets in lesions of *Candida* endocarditis (34). These observations were extended to a demonstration of yeast adherence to thrombin-induced fibrin-platelet matrices (39). Since vascular injury resulted in the deposition and the aggregation of platelets on exposed ECM, the hypothesis that they can enhance yeast adherence to subendothelial ECM was recently examined. Indeed, it was shown that platelets provide a very adhesive surface for *C. albicans* (Fig. 7) (34, 56). Moreover, denudation of the subendothelial ECM induced the adherence and aggregation of platelets, and yeasts were subsequently observed to adhere to these activated platelets (34).

Despite these promising results, there has been only limited investigation of the molecular basis of the interaction between fungi and platelets. To identify the potential yeast adhesins and the platelet receptors involved in this phenomenon, Mahaza et al. (personal communication) developed the following experimental approaches: (i), incubation of Nonidet P40-extracts of surface-iodinated platelets with *C. albicans* blastoconidia; and (ii), incubation of DTT-extract of ^{125}I-labeled germ tubes with platelets, both followed by SDS-PAGE and autoradiography. These studies have shown that the complex GP IIb-IIIa, and probably thrombospondin, may be the platelet factors and that the putative fungal receptor was a 45-47 kDa protein.

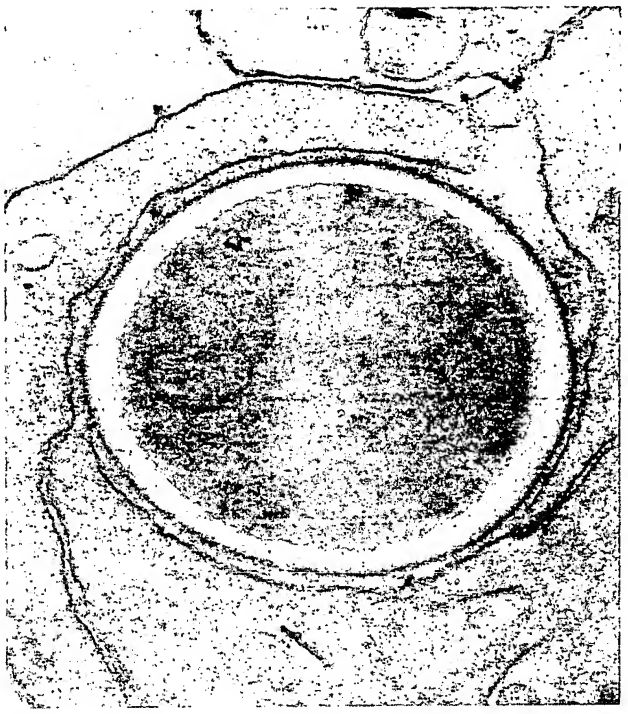


Figure 7. - Binding of germ tube to aggregated platelets, showing the close apposition of the platelet membrane and the fibrillar layer of the cell wall (x 10,000).

Fibrinogen is known to interact with various microorganisms (*Staphylococcus aureus, Bacteroides gingivalis* and *B. intermedius*, or the streptococci), and eucaryotic cells (particularly with platelets *via* the GP IIb/IIIa), and to play a major role in blood clot formation through its conversion to insoluble fibrin (14). Its binding to *C. albicans* was first studied by Bouali et al. (3) using an immunofluorescence assay with commercially available human fibrinogen. These studies demonstrated the existence of a fibrinogen binding factor (FBF) strongly associated with the surface of germ tubes and of filamentous forms of *C. albicans* (3, 4). The FBF appeared early during germ tube emergence and disappeared in ageing mycelia. By electron microscopy, fibrinogen binding was demonstrated associated with the flocculent outer layer (64). Post-embedding immunolabeling revealed fibrinogen binding in the inner part of the wall, over the plasma membrane and in the cytoplasm. Contrasting with the labeling of the mycelium cell wall, fibrinogen binding sites were not detected in any layer of the cell wall of blastoconidia, suggesting that if the FBF was present in blastoconidia, it exists under

an inactive form, unable to interact with fibrinogen. Both immobilized fibrinogen (fibrinogen conjugated to latex or gold particles) and free fibrinogen bound to the cell wall (64). Fibrinogen binding to *C. albicans* was also investigated in sections of tissues of experimentally infected mice: immunofluorescence assay showed that fibrinogen binds *in situ* to *C. albicans* and that FBF was excreted *in vivo* (Robert et al., unpublished observation).

Fibrinogen binding, like that of C3 fragments or fibronectin, appeared to correlate with pathogenicity, since less pathogenic or non-pathogenic *Candida* species did not interact with either fibrinogen (45) or fibrin (50). Moreover, it should be noted that fibrinogen bound to germ tubes with higher avidity than did albumin or transferrrin (45). Curiously, when fungal cells were treated with whole human plasma, fibrinogen bound over the entire surface of *C. albicans* blastoconidium-germ tube units, indicating different behaviour from that seen when the purified protein was tested alone (45).

In view of the importance of fibrinogen to the adherence of *C. albicans*, the biochemical characterization of the structures involved in this interaction was undertaken (2). Using ^{125}I-radiolabeled proteins, fibrinogen further purified by affinity chromatography to be free of fibronectin and laminin, and its plasmin degradation products (fragments D and E), we found that the binding sites on the fibrinogen molecule appeared to be located specifically in the D-domain. The binding was time-dependent, saturable and reversible. Scatchard analysis revealed an average of 6,000 binding sites per germ tube with a dissociation constant (K_d) of 5.2×10^{-8} M, similar to those reported for human cells. By SDS-PAGE and Western blotting, one component of 68 kDa was detected as a cell-wall binding protein.

Interactions with complement components:

Like other microorganisms and various human cells, *C. albicans* expresses surface molecules which link to cleavage products of the third component of complement (C3), iC3b and C3d. The presence of such receptors on *C. albicans* cell-wall surfaces was initially demonstrated by rosetting experiments between yeasts or hyphal forms and sheep erythrocytes coated with C3b, iC3b or C3d. *C. albicans* and *C. stellatoidea* were found to bind erythrocytes sensitized with iC3b or C3d but not with C3b, while less pathogenic *Candida* species did not present any receptor activity (15, 25). It is tempting to speculate that these receptors play an important role in the pathogenesis of *C. albicans* infections.

Biological consequences of these interactions have also been studied. Experiments showed that mycelial transformation correlated with increased expression of non-covalent binding sites for iC3b and significantly augmented the resistance of the organisms to phagocytosis (20, 21). Likewise, supplementation of the growth medium with glucose, which also induced an increased density of iC3b receptors on *C. albicans* cells, impeded phagocytosis (20, 21, 27) and augmented adherence to the host tissues (22). In contrast, phagocytosis was significantly augmented in the presence of Mo-1 (21), a monoclonal antibody (MAb) which recognizes the neutrophil complement receptor type 3, CR3 (also called CD11b/CD18) (17). Certain MAbs directed against CR3 inhibited the adherence of *C. albicans* to human endothelial cells *in vitro*. Another approach to study the functions of these receptors was the use of the mutant m-10: this mutant, which is characterized by its reduced ability to adhere to fibrin-platelet clots or to epithelial cells, and by its diminished virulence in rabbits and mice, presented a reduced density of iC3b binding sites compared to that of its parent wild-type strain (44).

Thus, the presence of iC3b receptors on *C. albicans* cells represents new virulence factor and raises the intriguing possibility that this lower eukaryote may mimic mammalian proteins in order to subvert the defenses of the host. Further support for this hypothesis has been provided by immunochemical and biomedical experiments.

iC3b receptors and molecular mimicry of integrins:

Integrins comprise a large family of cell surface receptors responsible for cell-cell and cell-matrix adhesion (28). These receptors consist of transmembrane heterodimers with two distinct non-covalently linked α and β subunits. There are at least 10 different integrin α subunits apparently unique for each receptor and 3 distinct integrin β subunits. For simplicity, integrins are divided into three classes called β_1, β_2 and β_3 on the basis of their β subunit. In most of them, including CR3, the α chain recognizes the amino acid sequence RGD (Arg-Gly-Asp), which is embedded in numerous unrelated matrix components (49).

Molecular mimicry of the neutrophil CR3, an integrin β_2 class of 165 kDa-MW for the α chain and 95 kDa for the β chain, has been extensively investigated. By immunofluorescence assay, several MAbs directed against α chain determinants of the CR3, OKM-1 (16, 20, 21), Mo-1 (15, 21, 26), M 1/70 (21) or M 522 (16), were found to bind specifically to the surface of *C. albicans*. In contrast, MAbs reacting with the β chain of CR3 (16), or recognizing the CR1 (CD 35), which links to C3b or the CR2 (CD 21) which links to C3dg/C3d (15, 16), failed to interact with *C. albicans*. Likewise, no reaction was observed with other MAbs against α chain determinants of the CR3 (Leu 15 and MN 41), suggesting that iC3b receptors on *C. albicans* and the α chain of the mammalian CR3 are antigenically related but not identical (16). Additionally, certain MAbs like OKM-1 were able to inhibit the attachment of iC3b-coated erythrocytes to *C. albicans* (15, 22).

In contrast to iC3b binding to the mammalian CR3, the attachment of iC3b to *C. albicans* did not

require the presence of divalent cations (16). Using radiolabeled iC3b, Gilmore et al. (21) showed that iC3b binding to *C. albicans* was saturable, specific and readily reversible; the dissociation constant (K_d) of 0.4 x10^{-6} M was similar to that described for the binding of iC3b to the neutrophil CR3 (21). Finally, Eigentler et al. (16) showed that *C. albicans* can bind a peptide containing the sequence RGD and that this binding interferes with iC3b binding to *C. albicans* pseudohyphae.

Biochemical studies were performed in order to identify the candidal receptors for iC3b. OKM-1 coupled to CNBr-activated Sepharose, immunoprecipitated a molecule of 130 kDa concomittant with minor bands of 50 and 100 kDa from iodinated surface material of *C. albicans* cells (16). Another MAb (BU-15), directed against the α chain of another β_2 integrin, p150/95, reacted in affinity chromatography with a 185-kDa protein of *C. albicans* cytosolic extract under non-reducing conditions, and with three proteins of 70, 67 and 55 kDa under reducing conditions (26). Likewise, Western blotting with a human serum that blocked iC3b rosetting, revealed components of similar MW (68 to 71, 55 and 50 kDa) in the parent wild-type strain of the mutant m-10 (44).

In addition, Mo-1 (22, 27) and BU-15 (26) disclosed, in Western blotting, a protein of 165 ± 15 kDa in membrane and cytosolic extracts of *C. albicans*, which is consistent with the published MW of the α chain of the mammalian CR3. BU-15 was also used by Hostetter and Kendrick (26) to screen a cDNA library of *C. albicans*, allowing the isolation of three immunoreactive clones containing Eco R1 fragments of 1.1, 3.8 and 3.9 kbp. These clones directed the synthesis of fusion proteins, which also reacted specifically with Mo-1. The amino acid sequence deduced from the 1.1 kbp fragment showed similarities with CR3 and p150/95.

Based upon these antigenic, structural and functional homologies, it seems that the iC3b receptors of *C. albicans* are members of the β_2 integrin family. Interestingly, molecular mimicry of β_1 class integrin has also been suggested for *C. albicans*. Using an antibody against a synthetic peptide corresponding to the COOH-terminal domain of the chicken β_1 subunit, Marcantonio and Hynes (41) showed, in immunoblotting, the presence of a cross-reactive protein of 95 kDa in membrane extract of *C. albicans*, while no band was detected for *S. cerevisiae.*

Characteristics of C. albicans C3d receptors:

Initially decribed by Heidenreich and Dierich (25), and latter confirmed by Edwards et al. (15), the presence of C3d receptors on *C. albicans* was further investigated by the group of Calderone.

As determined by rosetting experiments, receptor activity appeared to be expressed on germ tubes and pseudohyphae, but not on yeast forms of *C. albicans* (6, 15). Rosetting was markedly reduced by prior heating or treatment of the organisms with trypsin or pronase (6). A lectin-like interaction could be ruled out, since none of the oligosaccharides tested could affect the rosetting (6).

C3d binding structures were then purified from pseudohyphal extracts by ion exchange chromatography followed by affinity chromatography on immobilized C3d (6). Analysis of the eluate by SDS-PAGE and Western blotting with an anti-*C. albicans* MAb, which inhibits the rosetting, led to the identification of two mannoproteins of 62 and 70 kDa (6). After high-performance liquid chromatography, receptor activity appeared to be associated with the 60-kDa component (38). This glycoprotein contains equimolar amounts of glucose and mannose (38). As for the mammalian CR2, the carbohydrate moiety of this glycoprotein was not involved in the recognition process, but seemed to confer stability to the molecule (6).

More recently, the C3d binding component was purified from culture supernatant by isoelectric focusing (52). The purified protein presented a pI of 3.9 to 4.1 and was able to inhibit the attachment of C3d-coated erythrocytes to pseudohyphae. When analyzed by SDS-PAGE, this component migrated as a single band of 50 kDa in non-reducing gels, and as a doublet of 55 and 60 kDa in the presence of reducing agent. However, as indicated by Western blotting and concanavalin A staining, this component was mannoproteinic, and its treatment by endoglycosidase F resulted in complete conversion of the doublet into a broad band of 45 kDa.

Work is needed to elucidate the relationships between these different proteins. Nevertheless, some results suggest the absence of homology with the CR2 from B lymphocytes: (i), the lack of reactivity of *C. albicans* cell-wall surface in immunofluorescence staining with anti-CR2 MAbs (16, 21); (ii), the low pI of the candidal C3d receptor (52); and (iii), the amino acid composition of the purified protein, which differed significantly from that of the CR2, in that its glutamic acid content was high which might explain the low pI of the protein (52). Moreover, although considerable attention has been focused on defining the C3d binding structures on *C. albicans*, the functional significance of such receptors remains uncertain since the avirulent mutant m-10 and its parent wild-type strain exhibit similar abilities to bind C3d-coated erythrocytes (44).

CONCLUSION

The most intriguing aspect of the molecular organization and function of *C. albicans* adhesins is the similarities between these receptors and integrins on human cells. The belief that these adhesins are members of the integrin superfamily (16, 26), is supported by several observations. (i) *C. albicans* expresses surface receptors which interact with a wide variety of host proteins, such as fibronectin, laminin,

collagen, fibrinogen, iC3b and C3d. (ii) Like mammalian CR3, which links to iC3b and to a lesser extent to fibrinogen, the candidal adhesins are not completely specific, but present broad reactivity for multiple ligands: indeed, SDS-PAGE and ligand blotting techniques realized with fibrillar adhesin extract disclosed that the 68 and 60 kDa components reacted with laminin, fibrinogen and C3d, indicating their multifunctional potentialities (62); likewise, proteins of similar MWs were detected under reducing conditions as iC3b (26, 44) and C3d (6, 38) receptors. (iii) The iC3b receptor of *C. albicans* has an antigenic relationship to CR3, and preliminary results have shown similarities in the amino acid sequences (26) and RGD-peptide recognition of these two molecules (16). Homologies with other receptors have not yet been reported.

The main question concerning these interactions with host proteins is what are their exact implications *in vivo*. The hypothesis which states that fixation of host proteins represents mechanisms of host tissue adherence (Fig. 8) and of resistance to phagocytosis, has received much support during the last few years. However, there is not sufficient evidence to prove this

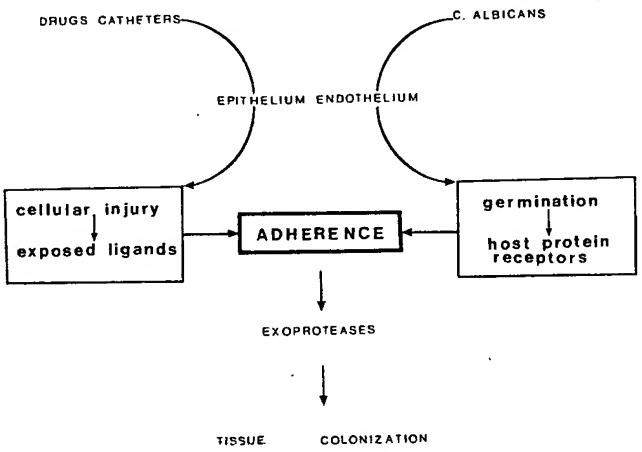


Figure 8. - Model for *C. albicans* adherence. Cellular injury caused by drugs or trauma (e.g. catheterization) exposes basement membrane ligands to contact with germinated yeasts expressing host protein receptors, resulting in adherence. Synthesis of exoproteases enables yeasts to penetrate to deeper layers and to colonize tissue.

hypothesis, since the influence of environmental pressure on receptor expression is unknown. Nevertheless, we can reasonably assume that the ability to interact with host proteins, together with molecular mimicry of mammalian adhesion molecules, constitutes a basis for the selection of a population of potential pathogens. From this point of view, there is no doubt that the isolation and characterization of *C. albicans* adhesins will lead to new therapeutic approaches based on the use of drugs (antibodies, ligand analogues,...) that block adherence and, therefore pathogenesis, in experimental models of infection.

Another question arises from the present concept of fungal integrins: since integrins consist of transmembrane heterodimers that interact with the cell matrix and cytoskeleton, their transport through the cell wall and their expression on its surface, as well as their relations with other cell wall components like glucans and mannans, remain to be determined. Until we have a better understanding of this major receptor system, we propose that the term "fungal cell adhesion molecules" (F. CAM) be used to refer to these proteins. The next decade will certainly see extensive investigation of these fungal molecules.

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